PROCEEDINGS OF THE YEREVAN STATE UNIVERSITY

Chemistry and Biology 2020, **54**(2), p. 99–104

Biology

BOVINE SERUM ALBUMIN FLUORESCENCE QUENCHING BY TANNIC ACID IN DIMETHYLSULFOXIDE CONTAINING SOLUTIONS

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Bovine serum albumin (BSA) interaction with tannic acid (TA) has been studied in dimethylsulfoxide (DMSO) aqueous solutions at different temperatures (293 and 303 *K*). To find out the fluorescence quenching mechanism of BSA in the presence of TA, the fluorescence data were analyzed according to the modified Stern-Volmer equation based on the approach of the existence of a "sphere of action" (a type of apparent static quenching). The values of apparent static and bimolecular quenching constants were calculated. The effect of DMSO and temperature on BSA–TA interactions is explained on the basis of structural changes in the "sphere of action" of the fluorophore due to the possible inclusion of DMSO molecules in this sphere.

https://doi.org/10.46991/PYSU:B/2020.54.2.099

***Keywords*:** bovine serum albumin, tannic acid, modified Stern-Volmer equation, dimethylsulfoxide, fluorescence, sphere of action.

Introduction. Tannic acid (TA) belongs to the class of plant polyphenols. The molecular structure of TA (chemical formula $C_{76}H_{52}O_{46}$, which corresponds to decagalloyl glucose) is presented in Fig. 1. Due to its polyphenolic structure, TA possesses antioxidant, antibacterial, anti-enzymatic and anti-mutagenic activity. It is used in preventive medicine, food and wine processing [1–3].

Serum albumins, especially human (HSA) and bovine (BSA), are commonly used as model proteins in biopharmaceutical studies. One of the most important biological functions of albumins is their ability to carry drugs, endogenous and exogenous substances [4–7]. BSA is a single chain globular protein comprising 582 amino acid residues. The tertiary structure of BSA is composed of three homologous domains (I, II, III), each of which in turn is divided into two subdomains (A and B) [8]. Due to the existence of phenylalanine (Phe), tyrosine (Tyr) and tryptophan (Trp) amino acid residues, the protein exhibits UV/Vis and fluorescence properties. BSA has two Trp residues that possess intrinsic fluorescence: Trp-134 in the first domain and Trp-212 in the second domain. The binding capacity of proteins depends on environmental characteristics such as temperature, ionic strength, pH and

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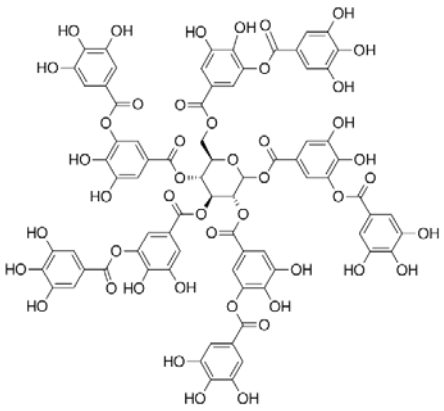
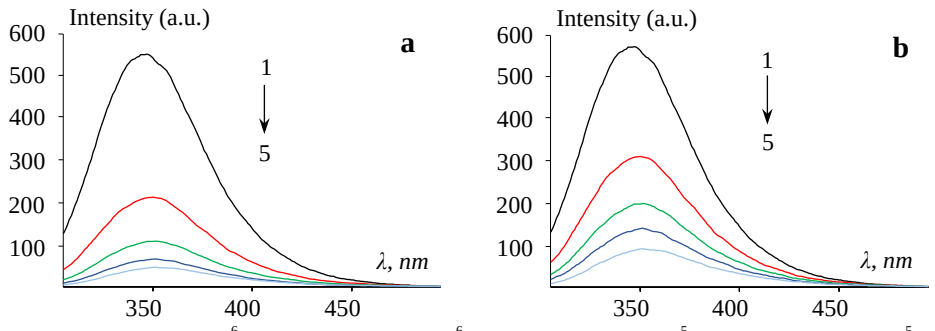


Fig. 1. Molecular structure of tannic acid.

composition of the solvent. As it was shown in [9], the preferential interaction of protein (HSA) with solvent components in dimethylsulfoxide (DMSO) containing binary aqueous solvents depends on the content of DMSO. At low DMSO concentrations, serum albumin undergoes preferential hydration, but at higher DMSO concentrations, preferential solvation of the protein by DMSO molecules occurs and a hydrophobic layer forms around the protein molecule. Fluorescence spectroscopy is a powerful method to study the interaction and structural changes of proteins due to its high sensitivity, selectivity and convenience. For biological macromolecules (DNA, proteins and etc.), the fluorescence measurements can provide reliable information on ligand binding thermodynamics, kinetics and mechanism [7, 10–12]. In the present paper, the interaction between TA and BSA in the presence of DMSO has been studied using fluorescence spectroscopy.

Materials and Methods. Fatty acid free (0.005%) BSA, TA and DMSO were purchased from "Sigma Aldrich" (USA). Solution of TA was prepared in double distilled water, and solution of BSA was prepared using physiological solution purchased from Likvor Pharmaceuticals (Armenia). The concentration of BSA (0.4 *mg*/*mL*) was determined spectrophotometrically by absorption at λ=280 *nm* and the extinction coefficient of 43.89 $M^{-1}cm^{-1}$ [13]. The concentration of DMSO was 0.53 *mol*/*L*. Fluorescence spectra were recorded on a Varian Cary Eclipse spectrophotometer (Australia) equipped with a circulating water thermostat (Lauda 100) in the λ=260–500 *nm* range at an excitation wavelength of 280 *nm*. The width of the excitation and emission slits was 5 *nm*. Quartz cells with *l*=1 *cm* were used for measurements. The sample temperature was kept constant throughout the experiment. The results were analyzed using the ORIGIN 8.0 software.

Results and Discussion. The fluorescence spectra of BSA in the presence of TA in aqueous and DMSO–aqueous solutions are presented in Fig. 2.



1 – [TA]=0; 2: [TA]=3.30·10^{-6} *mol/L;* 3 – [TA]=6.70·10^{-6} *mol/L;* 4 – [TA]=1.00·10^{-5} *mol/L;* 5 – [TA]=1.33·10^{-5} *mol/L*

Fig. 2. The fluorescence spectra of BSA in the presence of TA at various concentrations in aqueous (a) and DMSO–aqueous (b) solutions. BSA – 0.4 *mg*/*mL*, DMSO – 0.53 *mol*/*L*, *T*=303 *K*.

These spectra are characterized by a strong emission band at λ_{max}=347 *nm*, which decreases and shifts toward longer wavelengths ($\Delta\lambda$=8 *nm*) with the increase of TA concentration. These results show that the polarity of the fluorophore (Trp residues) microenvironment changes, becoming more polar. In the presence of DMSO, the quenching of BSA fluorescence is less effective.

Quenching data are usually presented as Stern–Volmer plots: F_0/F versus $[Q]$ (see Eq. 1). That is why F_0/F is expected to be linearly dependent on the concentration of the quencher. A linear Stern–Volmer plot is generally indicative of homogeneous quenching (static or dynamic). To find out the mechanism of BSA fluorescence quenching in the presence of TA, the fluorescence data were analyzed according to the Stern–Volmer equation:

$$\frac{F_0}{F} = 1 + k_q\tau_0[Q] = 1 + K_{SV}[Q], \quad (1)$$

where F_0 and F represent the fluorescence intensities in the absence and presence of a quencher; k_q is the bimolecular quenching rate constant; K_{SV} is the Stern–Volmer constant; τ_0 is the average lifetime of the fluorophore in the absence of a quencher, and $[Q]$ is the concentration of the quencher [14]. In many cases, a fluorophore can be quenched by both static (complex formation in the ground state) and dynamic (collisions in the excited state) mechanisms by the same quencher. In this case, the characteristic feature of the Stern–Volmer plot has an upward curvature. In other cases, an upward curvature indicates the existence of a "sphere of action" (a type of apparent static quenching), within which quenching occurs when the quencher approaches the excited fluorophore, but they do not actually form a ground state complex.

The simplest binding model of a ligand with a bio-macromolecule and complex formations in the ground state can be described by the following equilibrium: $F+Q \rightleftarrows [FQ]$, where F is the fluorophore, Q is the quencher and $[FQ]$ is the non-fluorescent complex of them. This situation can be characterized by the modified Stern–Volmer equation:

$$\frac{F_0}{F} = (1 + K[Q])\exp([Q]VN), \quad (2)$$

where K is the association constant; V is the volume of the sphere, and N the Avogadro's constant. Eq. 2 can be interpreted as a nonlinear generalization of the Stern–Volmer equation if in the latter one notes that for $K[Q]$ small enough $(1 + K[Q]) \approx \exp(K[Q])$, which is equivalent to $\exp([Q]VN)$ in Eq. 2. So, Eq. 1 transforms into the modified Stern–Volmer equation of the following form:

$$\frac{F_0}{F} = \exp(K[Q]), \text{ hence } \ln\frac{F_0}{F} = K[Q]. \quad (3)$$

As can be seen from Fig. 3a, the Stern–Volmer plots deviate from linearity with an upward curvature, concave towards the y-axis in both aqueous and DMSO–aqueous solutions, but in the presence of DMSO, the curvature is less pronounced. At higher temperatures (30°C), the trend of the dependences does not change, however, the quenching of the fluorophore is less effective.

These dependences are not due to the combined (static and dynamic) quenching mechanism since the modified Stern–Volmer plots also deviate from linearity.

To interpret the data on BSA fluorescence quenching by TA, the modified Stern–Volmer equation (Eq. 3) describing "sphere of action" model was used. The

apparent static (K_{app}) and bimolecular quenching (k_q) constants were determined using Fig. 3b and are presented in the Table. k_q was calculated using the BSA lifetime (τ_0) of approximately 5 *ns* [15] in the absence of a quencher. The values of K_{app} in aqueous solutions were higher than in DMSO–aqueous solutions, which can be explained by the thickening of the "sphere of action" of fluorophore due to the inclusion of DMSO molecules in this sphere. At low concentrations (0.53 *mol/L*) DMSO forms strong complexes with H_2O via hydrogen bonding [16], therefore the probability of penetration and binding of DMSO molecules in the "sphere of action" is very high. The effect of temperature on the stability of the formed complexes in aqueous and DMSO containing solutions is expressed in the same pattern: at higher temperatures the stability of these complexes decreases. In the presence of DMSO these complexes are more stable due to structuring of the "sphere of action".

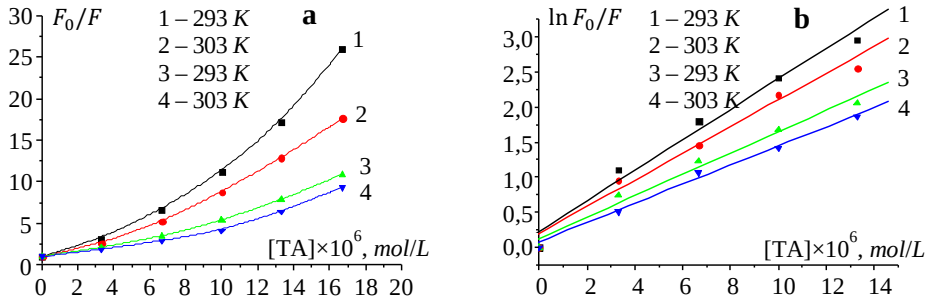


Fig. 3. Stern–Volmer (a) and modified Stern–Volmer (b) plots for BSA–TA in aqueous (1, 2) and DMSO–aqueous (3, 4) solutions at different temperatures.

Apparent static (K_{app}) and bimolecular quenching (k_q) constants for the BSA–TA interaction

Constant	H_2O		H_2O–DMSO	
	20°C	30°C	20°C	30°C
$K_{app}\times10^{-5}$ (M^{-1})	2.15±0.02294	1.97±0.01668	1.51±0.09671	1.36±0.00512
$k_q\times10^{13}$ ($M^{-1}\,s^{-1}$)	4.30	3.95	3.02	2.72

Conclusion. The interaction of BSA with TA in DMSO–aqueous solutions was evaluated at different temperatures by measuring the intensity of intrinsic fluorescence of BSA tryptophan residues (Trp-134 and Trp-214). The quenching of BSA fluorescence in the presence of TA is due to the interactions in the "sphere of action". The quenching property of TA depends on the changes in the structure of the "sphere of action" under the influence of the temperature and the inclusion of DMSO molecules in this sphere.

This work was supported by SC MESCS of RA, in the frame of protection financing of Basic Laboratory of the Chair of Physical and Colloids Chemistry of YSU and research project 18 RF-020.

Received 01.04.2020
Reviewed 08.07.2020
Accepted 27.07.2020

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ՑՈՒԼԻ ՇԻՃՈՒԿԱՅԻՆ ԱԼԲՈՒՄԻՆԻ ՖԼՈՒՈՐԵՍՑԵՆՑԻԱՅԻ ՄԱՐՈՒՄԸ ԴԱԲԱՂԱԹԹՎԻ ՆԵՐԿԱՅՈՒԹՅԱՄԲ ԴԻՄԵԹԻԼՍՈՒԼՖՕՔՍԻԴ ՊԱՐՈՒՆԱԿՈՂ ԼՈՒԾՈՒՅԹՆԵՐՈՒՄ

Ուսումնասիրվել է ցուլի շիճուկային ալբումինի (ՑՇԱ) փոխազդեցությունը դաբաղաթթվի հետ (ԴԹ) դիմեթիլսուլֆօքսիդի (ԴՄՍՕ) ջրային լուծույթներում տարբեր ջերմաստիճաններում (293 և 303 Կ): ԴԹ-ի ներկայությամբ ՑՇԱ-ի ֆլուորեսցենցիայի մարման մեխանիզմը պարզելու համար տվյալները վերլուծվել են Շտերնի–Ֆոլմերի ձևափոխված հավասարման օգնությամբ, որի հիմքում ընկած է «գործողության թաղանթի» առկայության մոտեցումը: Հաշվարկվել են թվացյալ ստատիկ մարման և երկմոլեկուլային բախման հաստատունների արժեքները: Ջերմաստիճանի և ԴՄՍՕ-ի ազդեցությունը ՑՇԱ–ԴԹ փոխազդեցության վրա բացատրվել է ֆլուորոֆորի «գործողության թաղանթի» կառուցվածքային փոփոխություններով՝ պայմանավորված այդ թաղանթ ԴՄՍՕ-ի մոլեկուլների հնարավոր ներգրավմամբ:

К. Р. ГРИГОРЯН, А. А. ШИЛАДЖЯН, В. А. ОГАНЕСЯН

ТУШЕНИЕ ФЛУОРЕСЦЕНЦИИ БЫЧЬЕГО СЫВОРОТОЧНОГО АЛЬБУМИНА В ПРИСУТСТВИИ ДУБИЛЬНОЙ КИСЛОТЫ В ДИМЕТИЛСУЛЬФОКСИД СОДЕРЖАЩИХ РАСТВОРАХ

Изучено взаимодействие бычьего сывороточного альбумина (БСА) с дубильной кислотой (ДК) в водных растворах диметилсульфоксида (ДМСО) при различных температурах (293 и 303 *K*). Для выяснения механизма тушения флуоресценции БСА в присутствии ДК данные флуоресценции были проанализированы в соответствии с модифицированным уравнением Штерна–Фольмера, в основе которого лежит подход о существовании “сферы взаимодействия”. Рассчитаны константы кажущегося статического тушения и бимолекулярного столкновения. Влияние температуры и ДМСО на взаимодействия БСА–ТА объясняется структурными изменениями “сферы взаимодействия” за счет возможного включения молекул ДМСО в эту сферу.